

IN THE SPECIFICATION

Please amend the table on page 28, lines 14-22, as follows:

Table 1. Oligonucleotide primers used in PCR.

Primer	Primer sequence	Size
Tbp1	thr-glu-asn-lys-lys-ile-gly-glu (SEQ ID NO:68) 5'GGAAGCTTACT-GAA-AAT-AAA-AAA-ATC-GAA-GAA * (SEQ ID NO:22)	32 mer
primer left	5'GGAATTCCTCGTCCTGTGGATC** (SEQ ID NO:23)	22 mer
primer right	5'GTGAATTCGGCGTAGAGGATC** (SEQ ID NO:24)	22 mer

* - the underlined sequence is the *Hind*III site.

** - the underlined sequence is the *Eco*RI site.

P. 7,
Please amend the paragraph, lines 15-16, as follows:

Figure 12 is a peptide alignment between *P. haemolytica* A1 Tbp1 and
TonB-dependent outer membrane receptors of *E. coli* (SEQ ID NO:16-20, 53-67).

Please delete the Sequence Listing and replace it with the Substitute
Sequence Listing included herewith.

REMARKS

It has come to the Applicants' attention that the sequence listing submitted
July 3, 2000 contains inadvertent errors. The sequences were correctly designated in the
specification and the figures. It is respectfully submitted that this submission does not
require any additional search or examination, and is directed only to the correction of
formal matters.

For SEQ ID NO:1, the nucleotide "n" at position 2544 is missing from the
sequence listing. Figure 21 and SEQ ID NO:2 provide the corresponding protein

sequence in which the amino acid at position 848 is Gly. Codons for Gly are *gga*, *ggc*, *ggg* or *ggg*. Thus, to one of skill in the art, the nucleotide at position 2544 can be a,c,t or g or "n." For SEQ ID NOS: 8 and 11, the organism is *N. gonorrhoeae*, as correctly listed in Figures 4 and 9, respectively. For SEQ ID NOS: 9 and 12, the organism is *N. meningitidis*, as correctly listed in Figures 4 and 9, respectively. For SEQ ID NOS: 14 and 15, the organism is *A. pleuropneumoniae*, as correctly listed in Figure 10. For SEQ ID NOS: 16-20, the length of the sequences is 7 amino acids as set forth in Figure 12. For SEQ ID NOS:23 and 24, the sequences are primers as listed on Table 1, p. 28 and corrected in the substitute sequence listing as Artificial Sequences. For SEQ ID NOS:25-27, 30, and 32-37, the sequences are primers as listed on Table 4, p47-48 and corrected in the substitute sequence listing as Artificial Sequences. For SEQ ID NOS:28, 29, and 31, the sequences are primers as listed on Table 4, p. 47 and corrected in the substitute sequence listing as Artificial Sequences and correct designation of inosine bases. For SEQ ID NOS:46-52, the sequences are primers as listed on Table 10, p63-64 and corrected in the substitute sequence listing as Artificial Sequences

Applicants submit herewith a substitute Sequence Listing in computer and paper form, in accordance with 37 C.F.R. §1.821-1.825. The content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same and do not include new matter.

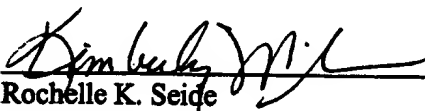
The specification has been amended to insert sequence identifiers and replace the sequence listing with the substitute sequence listing included herewith. No new matter has been introduced as a result of the amendments. Attached hereto is a page captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" indicating

the changes made to the claims. Material added is double underlined and material deleted is in brackets.

In view of the foregoing, entry of this amendment is respectfully requested.

Respectfully submitted,

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Attorneys for Applicants



MARKED UP VERSION OF TO SHOW CHANGES MADE

IN THE SPECIFICATION

The table on page 28, lines 14-22, has been amended as follows:

Table 1. Oligonucleotide primers used in PCR.

Primer	Primer sequence	Size
Tbp1	thr-glu-asn-lys-lys-ile-gly-glu 5' <u>GGAAGCTT</u> ACT-GAA-AAT-AAA-AAA-ATC-GAA-GAA * (SEQ ID NO:68) (SEQ ID NO:22)	32 mer
primer left	5' <u>GGAATTC</u> CGTCCTGTGGATC** (SEQ ID NO:23)	22 mer
primer right	5' <u>GTGAATTC</u> GGCGTAGAGGATC** (SEQ ID NO:24)	22 mer

* - the underlined sequence is the *Hind*III site.

** - the underlined sequence is the *Eco*RI site.

The paragraph, lines 15-16, has been amended as follows:

Figure 12 is a peptide alignment between *P. haemolytica* A1 Tbp1 and
TonB-dependent outer membrane receptors of *E. coli* (SEQ ID NO:16-20, 53-67).

The Sequence Listing has been replaced with the Substitute Sequence
Listing included herewith .



A34762 - 021645.0105
PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant : Lo et al.
Serial. No : 08/753,750 Examiner: A. Harris
Filed : November 29, 1996 Group Art Unit: 1642
For : TRANSFERRIN BINDING PROTEINS OF PASTEURELLA
HAEMOLYTICA AND VACCINES CONTAINING SAME

AMENDMENT UNDER 37 C.F.R. § 1.312 AND SUBMISSION OF
FORMAL DRAWINGS

October 1, 2002

EXPRESS MAIL NO: ET 346773367US

BOX ISSUE FEE

Assistant Commissioner for Patents
Washington, D.C. 20231

Attention: Official Draftsperson

Sir:

In response to the Notice of Allowability dated July 2, 2002, Applicants submit herewith thirty-two (32) sheets of Formal Drawings. These Drawings correspond to Figures 1-11, 13-20, 23, and 25-29. Please substitute these drawings for the drawings previously filed.

Applicants respectfully request consideration of the the following amendments to the specification.

IN THE SPECIFICATION

Please amend the paragraph on page 6, lines 32-33 as follows:

NY02:408520.1

H4
Figure 1A-1B is a schematic diagram of the PCR procedure (a), and the 0.8 kb PCR product amplified by Tbp1 primer and primer left (b);

Please amend the paragraph on page 6, line 35 as follows:

H5
Figure 3A-3E is a preliminary nucleotide sequence of *P. haemolytica* *tpbA* and *tpbB*, Figure 3A-3B shows the sequence of *tpbB* and Figure 3C-3E shows the sequence of *tpbA*.

Please amend the paragraph on page 7, lines 8-9 as follows:

H6
Figure 9A-9C shows an alignment of the amino acid of Tbp1 of *P. haemolytica* A1, (PHTBP) and the Tbp1 of *N.gonorrhoeae* (NGTBP1) and *N. meningitidis* (NM1);

Please amend the paragraph on page 7, lines 10-11 as follows:

H7
~~H7~~
Figure 10A-10B shows an alignment of the amino acid of Tbp1 of *P. haemolytica* A1, (PHTBP) and the *A. pleuropneumoniae* serotype 1 and 7 TfbA proteins (APL, APL7);

Please amend the paragraph on page 7, lines lines 25-26 as follows:

H8
Figure 17A-17C is an immunoblot showing isolation of receptor proteins with transferrin affinity columns;

Please amend the paragraph on page 7, lines 27-29 as follows:

H9
Figure 18A and 18B are immunoblots showing immunological analysis of receptor proteins from different serotypes of *P. haemolytica* from bovine, sheep, and goats, where Panel A is with anti-TbpB serum and Panel B is with anti-TbpA serum;

Please amend the paragraph on page 7, lines 30-31 as follows:

H10
Figure 19A-19C are blots showing the binding of labelled transferrin and anti-receptor antibody by intact cells;

Please amend the paragraph on page 7, lines 32-35 as follows:

H11
Figure 20A-20B is a map of the *P. haemolytica* *tbp* operon (Top) and *P. haemolytica* *tbp* operon (Top) and regulatory sequences (Bottom); *tbpA* and *tbpB* are the genes encoding for TbpA and TbpB, respectively; p, is the putative promoter region preceding *tbpB* and denoted as -35 and -10 sites at the bottom;

Please amend the paragraph on page 8, lines 8-9 as follows:

H12
Figure 26A-26B are blots showing silver stain (Panel A), and western blot (Panel B) studies with anti-TbpA and anti-TbpB antisera from *P. haemolytica* serotype A1;

Please amend the paragraph on page 8, lines 12-14 as follows:

H13
Figure 28A-28B shows a gel with restriction endonuclease digestion patterns of PCR-amplified *tbpA* (Panel A) and *tbpB* (Panel B) genes from *P. haemolytica* and *P. trehalosi* strains; and

Please amend the paragraph on page 8, lines 15-16 as follows:

H'4

Figure 29A-29B is a gel showing PCR amplification of variable segments of the *tbpA* (Panel A) and *tbpB* (Panel B) genes.

Please amend the paragraph on page 76, line 2 as follows:

H'5

Figure 1A-1B. Result of PCR analysis

Please amend the paragraph on page 76, lines 13-14 as follows:

H'6

Figure 3A-3E. Preliminary nucleotide sequence of *P. haemolytica* *tbpA* and *tbpB*. Putative signal sequence cleavage sites are indicated by an arrow. The start codon (ATG) is underlined.

Please amend the paragraph on page 76, lines 32-36 as follows:

H'7

Figure 9A-9C. Alignment of the amino acid Tbp1 of *P. haemolytica* A1 (PHTBP) and the Tbp1 of *N. gonorrhoeae* (NGTBP1) and *N. meningitidis* (NM1). The numbers to the right indicate amino acid positions. Asterisks indicate positions of complete alignment, dots indicate similar amino acid residues. Gaps were introduced to maximize sequence alignment and are indicated by dashes (-).

Please amend the paragraph on page 77, lines 1-4 as follows:

H'8

Figure 10A-10B. Alignment between *P. haemolytica* A1 Tbp1 (PHTBP1) and the *A. pleuropneumoniae* serotype 1 and 7 TfbA proteins (APL, APL7). Asterisks indicate positions of complete identity in alignment, dots indicate similar amino acid

418 residues. Gaps were introduced to maximize sequence alignment and are indicated by dashes (-).

Please amend the paragraph on page 78, lines 1-9, as follows:

419 **Figure 17A-17C. Isolation of receptor proteins with transferrin affinity columns,**
Affinity isolation experiments were performed with iron-deficient total membranes prepared from *P. haemolytica* strain h44 (top panel), h173 (middle panel) and h175 (bottom panel). Experiments were performed with bovine transferrin-Sepharose (lane A and B), ovine transferrin-Sepharose (lane C), caprine transferrin-Sepharose (lane D) or equine transferrin-Sepharose (lane E) using standard washing conditions (lanes B-e) or low salt washing conditions (lane A) as outlined in the methods section. The samples eluted with buffer containing 2M guanidine HCl were dialyzed, concentrated and aliquots analyzed by SDS-PAGE and silver staining as described in the methods section.

Please amend the paragraph on page 78, lines 10-17, as follows:

420 **Figure 18A-18B. Immunological analysis of receptor proteins from different**
serotypes of *P. haemolytica* from bovine, sheep and goats. Aliquots of purified receptor proteins from representative serotypes of *P. haemolytica* were subjected to SDS-PAGE, electroblotted and then probed with specific anti-TbpB serum (Panel A) or with anti-TbpA serum (Panel B) as described in the methods section. The following *P. haemolytica* strains of the indicated serotype were included in the methods analysis; Lane 1 - strain h44 (A1), Lane 2 - h173 (untypable), Lane 3 - h175 (A7), Lane 4 - h176 (A9),

420 Lane 5 - h100 (T4), Lane 6 - h106 (T10), Lane 7 - h107 (A11). The numbers on the left represent the molecular weights (X1000) of standard proteins.

Please amend the paragraph on page 78, lines 18-24, as follows:

421 **Figure 19A-19C.** Binding of labelled transferrin and anti-receptor antibody by intact cells. The indicated bacterial strains were grown under iron-limiting conditions, harvested by centrifugation and resuspended to a A_{600} of 1-2 in 50mM TrisHCl, 150mM NaCl, pH 7.5 buffer. A 5 μ l aliquot of the suspensions were applied to HA membrane, the membrane was dried, blocked and then exposed to blocking solution containing labelled transferrin (HRP-bTf) or antireceptor antibody (anti-TbpA, anti-TbpB). The latter membranes were washed and subsequently exposed to labelled second antibody prior to development with substrate.

Please amend the paragraph on page 78, lines 25-32, as follows:

422 **Figure 20A-20B.** Map of the *P. haemolytica* *tbp* operon (Top) and regulatory sequences (Bottom). *tbpA* and *tbpB* are the genes encoding for TbpA and TbpB, respectively; *p*, is the putative promoter region preceding *tbpB* and denoted as -35 and -10 sites at the bottom. A putative Fur box is represented as two arrows in opposite directions in the sequence at the bottom; *maseT* and *fis* are two ORFs flanking the *P. haemolytica* *tbp* operon encoding for proteins highly homologous to *E. coli* and *H. influenza* RNase transferase and factor-for-inversion stimulation proteins, respectively. Additionally, putative ribosomal binding site or Shine-Dalgarno (SD) consensus sequence, transcriptional start (Met), and stop codons (SC) are also bolded.

Please amend the paragraph on page 79, lines 16-22, as follows:

H23
Figure 26A-26B. Western blot cross-reactivity studies with anti-TbpA and anti-TbpB antisera from *P. haemolytica* serotype A1. Aliquots of affinity purified receptor proteins from the indicated strains of *P. haemolytica* and *P. trehalosi* were separated by SDS-PAGE and Silver stain (panel A) or subjected to Western blotting (panel B) as in Methods. Tbp proteins were identified by incubation with a mixture of anti-TbpB (1/1000) and anti-TbpA (1/1000) antisera as described in the methods section. The numbers on the left represent the molecular weight (X1000) of standard proteins in kilodaltons.

Please amend the paragraph on page 79, line 31, as follows:

H24
Figure 28A-28B. Restriction endonuclease digestion pattern of PCR amplified *tbpA* (Panel A) and *Tbp* (Panel B) genes from *P. haemolytica* and *P. trehalosi* strains. The *tbp* genes amplified by colony PCR from the indicated strains were digested with *Sau3A1* restriction endonuclease. The resulting digests were electrophoresed on a 7.5% polyacrylamide gel as described in Methods. The letters above the lanes indicate the source strain template DNA used in PCR while the letters on the left indicate the molecular weight standard in kilobases. Imaging was done with a Hewlett-Packard ScanJet IIp. In fig 29B, primer #s 397 and 400 from non conserved regions of *P. haemolytica tbpB* gene, were used in combination with opposing primers {#s 401 (5') and 199(3')} respectively.

Please amend the paragraph on page 80, lines 3-7, as follows:

H²⁵
Figure 29A-29B. PCR amplification of variable segments of the *tbpA* and *tbpB* genes. For *tbpA* gene, oligonucleotide primer #450 made from the deduced amino acid sequence from a hypervariable region of *tbpA* was used in combination with the 5' specific primer (#255) to amplify the gene segment from the various *P.haemolytica* strains. The products were then analysed on 1% agarose gel followed by staining with ethidium bromide.

REMARKS

The Applicants submit herewith thirty-two (32) sheets of formal drawings in order to correct the changes to the drawings required by the Notice of Draftspersons' Patent Drawing Review appended to the Office Action dated October 23, 2001.

The BRIEF DESCRIPTION OF THE DRAWINGS in the specification has been amended to correctly identify figures submitted herewith. No new matter has been introduced as a result of the amendments. Attached hereto is a page captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE"** indicating the changes made to the claims.

Respectfully submitted,

BAKER BOTTS L.L.P.

By: 

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Kimberly J. McGraw
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Attorneys for Applicants



SECTION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

The paragraph on page 6, lines 32-33 has been amended as follows:

Figure 1A-1B is a schematic diagram of the PCR procedure (a), and the 0.8 kb PCR product amplified by Tbp1 primer and primer left (b);

The paragraph on page 6, line 35 has been amended as follows:

Figure 3A-3E is a preliminary nucleotide sequence of *P. haemolytica* *tbpA* and *tpbB*. Figure 3A-3B shows the sequence of *tpbB* and Figure 3C-3E shows the sequence of *tpbA*.

The paragraph on page 7, lines 8-9 has been amended as follows:

Figure 9A-9C shows an alignment of the amino acid of Tbp1 of *P. haemolytica* *A1*, (PHTBP) and the Tbp1 of *N. gonorrhoeae* (NGTBP1) and *N. meningitidis* (NM1);

The paragraph on page 7, lines 10-11 has been amended as follows:

Figure 10A-10B shows an alignment of the amino acid of Tbp1 of *P. haemolytica* *A1*, (PHTBP) and the *A. pleuropneumoniae* serotype 1 and 7 TfbA proteins (APL, APL7);

The paragraph on page 7, lines 25-26 has been amended as follows:

Figure 17A-17C is an immunoblot showing isolation of receptor proteins with transferrin affinity columns;

The paragraph on page 7, lines 27-29 has been amended as follows:

Figure 18A and 18B are immunoblots showing immunological analysis of receptor proteins from different serotypes of *P. haemolytica* from bovine, sheep, and goats, where Panel A is with anti-TbpB serum and Panel B is with anti-TbpA serum;

The paragraph on page 7, lines 30-31 has been amended as follows:

Figure 19A-19C are blots showing the binding of labelled transferrin and anti-receptor antibody by intact cells;

The paragraph on page 7, lines 32-35 has been amended as follows:

Figure 20A-20B is a map of the *P. haemolytica* *tbp* operon (Top) and *P. haemolytica* *tbp* operon (Top) and regulatory sequences (Bottom); *tbpA* and *tbpB* are the genes encoding for TbpA and TbpB, respectively; p, is the putative promoter region preceding *tbpB* and denoted as -35 and -10 sites at the bottom;

The paragraph on page 8, lines 8-9 has been amended as follows:

Figure 26A-26B are blots showing silver stain (Panel A), and western blot (Panel B) studies with anti-TbpA and anti-TbpB antisera from *P. haemolytica* serotype A1;

The paragraph on page 8, lines 12-14 has been amended as follows:

Figure 28A-28B shows a gel with restriction endonuclease digestion patterns of PCR-amplified *tbpA* (Panel A) and *tbpB* (Panel B) genes from *P. haemolytica* and *P. trehalosi* strains; and

The paragraph on page 8, lines 15-16 has been amended as follows:

Figure 29A-29B is a gel showing PCR amplification of variable segments of the *tbpA* (Panel A) and *tbpB* (Panel B) genes.

The paragraph on page 76, line 2 has been amended as follows:

[Figure 1.] Figure 1A-1B. Result of PCR analysis

The paragraph on page 76, lines 13-14 has been amended as follows:

[Figure 3.] Figure 3A-3E. Preliminary nucleotide sequence of *P. haemolytica* *tbpA* and *tbpB*. Putative signal sequence cleavage sites are indicated by an arrow. The start codon (ATG) is underlined.

The paragraph on page 76 lines 32-36, has been amended as follows:

[Figure 9.] Figure 9A-9C. Alignment of the amino acid Tbp1 of *P. haemolytica* A1 (PHTBP) and the Tbp1 of *N. gonorrhoeae* (NGTBP1) and *N. meningitidis* (NM1). The numbers to the right indicate amino acid positions. Asterisks indicate positions of complete alignment, dots indicate similar amino acid residues. Gaps were introduced to maximize sequence alignment and are indicated by dashes (-).

The paragraph on page 77, lines 1-4, has been amended as follows:

[Figure 10.] **Figure 10A-10B.** Alignment between *P. haemolytica* A1 Tbp1 (PHTBP1) and the *A. pleuropneumoniae* serotype 1 and 7 TfbA proteins (APL, APL7). Asterisks indicate positions of complete identity in alignment, dots indicate similar amino acid residues. Gaps were introduced to maximize sequence alignment and are indicated by dashes (-).

The paragraph on page 78, lines 1-9, has been amended as follows:

[Figure 17.] **Figure 17A-17C.** Isolation of receptor proteins with transferrin affinity columns, Affinity isolation experiments were performed with iron-deficient total membranes prepared from *P. haemolytica* strain h44 (top panel), h173 (middle panel) and h175 (bottom panel). Experiments were performed with bovine transferrin-Sepharose (lane A and B), ovine transferrin-Sepharose (lane C), caprine transferrin-Sepharose (lane D) or equine transferrin-Sepharose (lane E) using standard washing conditions (lanes B-e) or low salt washing conditions (lane A) as outlined in the methods section. The samples eluted with buffer containing 2M guanidine HCl were dialyzed, concentrated and aliquots analyzed by SDS-PAGE and silver staining as described in the methods section.

The paragraph on page 78, lines 10-17, has been amended as follows:

[Figure 18.] **Figure 18A-18B.** Immunological analysis of receptor proteins from different serotypes of *P. haemolytica* from bovine, sheep and goats. Aliquots of purified receptor proteins from representative serotypes of *P. haemolytica* were subjected to SDS-PAGE, electroblotted and then probed with specific anti-TbpB serum (Panel A) or with anti-TbpA serum (Panel B) as described in the methods section. The following *P.*

haemolytica strains of the indicated serotype were included in the methods analysis; Lane 1 – strain h44 (A1), Lane 2 – h173 (untypable), Lane 3 – h175 (A7), Lane 4 – h176 (A9), Lane 5 – h100 (T4), Lane 6 – h106 (T10), Lane 7 – h107 (A11). The numbers on the left represent the molecular weights (X1000) of standard proteins.

The paragraph on page 78, lines 18-24, has been amended as follows:

[Figure 19.] **Figure 19A-19C.** Binding of labelled transferrin and anti-receptor antibody by intact cells. The indicated bacterial strains were grown under iron-limiting conditions, harvested by centrifugation and resuspend to a A_{600} of 1-2 in 50mM TrisHCl, 150mM NaCl, pH 7.5 buffer. A 5 μ l aliquot of the suspensions were applied to HA membrane, the membrane was dried, blocked and then exposed to blocking solution [containg] containing labelled transferrin (HRP-bTf) or antireceptor antibody (anti-TbpA, anti-TbpB). The latter membranes were washed and subsequently exposed to labelled second antibody prior to development with substrate.

The paragraph on page 78, lines 25-32, has been amended as follows:

[Figure 20.] **Figure 20A-20B.** Map of the *P. haemolytica* *tbp* operon (Top) and regulatory sequences (Bottom). *tbpA* and *tbpB* are the genes encoding for TbpA and TbpB, respectively; *p*, is the putative promoter region preceding *tbpB* and denoted as –35 and –10 sites at the bottom. A putative Fur box is represented as two arrows in opposite directions in the sequence at the bottom; *maseT* and *fis* are two ORFs flanking the *P. haemolytica* *tbp* operon encoding for proteins highly homologous to *E. coli* and *H. influenza* RNase transferase and factor-for-inversion stimulation proteins, respectively.

Additionally, [putatives] putative ribosomal binding site or Shine-Dalgarno (SD) consensus sequence, transcriptional start (Met), and stop codons (SC) are also bolded.

The paragraph on page 79, lines 16-22, has been amended as follows:

[Figure 26.] **Figure 26A-26B.** Western blot cross-reactivity studies with anti-TbpA and anti-TbpB antisera from *P. haemolytica* serotype A1. Aliquots of affinity purified receptor proteins from the indicated strains of *P. haemolytica* and *P. trehalosi* were separated by SDS-PAGE and Silver stain (panel A) or subjected to Western blotting (panel B) as in Methods. Tbp proteins were identified by incubation with a mixture of anti-TbpB (1/1000) and anti-TbpA (1/1000) antisera as described in the methods section. The numbers on the left represent the molecular weight (X1000) of standard proteins in kilodaltons.

The paragraph beginning on page 79, line 31 and ending on page 80, line 2, has been amended as follows:

[Figure 28.] **Figure 28A-28B.** Restriction endonuclease digestion pattern of PCR amplified *tbpA* (Panel A) and Tbp (Panel B) genes from *P. haemolytica* and *P. trehalosi* strains. The *tbp* genes amplified by colony PCR from the indicated strains were digested with Sau3A1 restriction endonuclease. The resulting digests were electrophoresed on a 7.5% polyacrylamide gel as described in Methods. The letters above the lanes indicate the source strain template DNA used in PCR while the letters on the left indicate the molecular weight standard in kilobases. Imaging was done with a Hewlett-Packard ScanJet IIP. In fig 29B, primer #s 397 and 400 from non conserved regions of [*P.*

hamolytica] *P. haemolytica* *tbpB* gene , were used in combination with opposing primers {#s 401 (5') and 199(3')} respectively.

The paragraph on page 80, lines 3-7, has been amended as follows:

[Figure 29.] **Figure 29A-29B.** PCR amplification of variable segments of the *tbpA* and *tbpB* genes. For *tbpA* gene, oligonucleotide primer #450 made from the deduced amino acid sequence from a hypervariable region of *tbpA* was used in combination with the 5' specific primer (#255) to amplify the gene segment from the various *P.haemolytica* strains. The products were then analysed on 1% agarose gel followed by staining with ethidium bromide.